

## AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

### **LISTING OF CLAIMS**

1. (Currently amended) An oral vaccine comprising, in an orally suitable formulation, at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the group consisting of isolated recombinant adhesion proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8, ~~and immunogenic fragments thereof~~, wherein said vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.

2. (Previously presented) The vaccine of Claim 1 wherein at least one of the recombinant protein fragments is emulsified in a water-in-oil emulsion.

3. (Previously presented) The vaccine according to Claim 2 wherein said emulsifying oil comprises organic oil.

4. (Previously presented) The vaccine according to Claim 3 wherein said emulsifying oil comprises palm oil.

5. (Previously presented) The vaccine according to Claim 2 wherein the proportion of water and oil in the emulsion is in the ratio of 1:2.

6. (Original) The vaccine according to Claim 2 wherein the proportion of water and oil in the emulsion is equal.

7. (Original) The oral vaccine according to Claim 2 mixed with a binding agent.

8. (Previously presented) The oral vaccine of Claim 7 wherein the binding agent comprises particulate feed material.

9. (Previously presented) The oral vaccine of Claim 8 wherein the binding agent comprises high viscosity carboxymethylcellulose.

10. (Original) The oral vaccine of Claim 1 comprising an immunologically effective dose of recombinant AHMA protein.

11. (Previously presented) The oral vaccine according to Claim 1 further comprising recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis*.

12. (Original) The vaccine of Claim 11 wherein the recombinant proteins are emulsified in a water-in-oil emulsion.

13. (Previously presented) The vaccine according to Claim 12 wherein said emulsifying oil comprises organic oil.

14. (Previously presented) The vaccine according to Claim 13 wherein said emulsifying oil comprises palm oil.

15. (Previously presented) The vaccine according to Claim 12 wherein the proportion of water and oil in the emulsion is in the ratio of 1:2.

16. (Original) The vaccine according to Claim 12 wherein the proportion of water and oil in the emulsion is equal.

17. (Original) An oral vaccine according to Claim 12 mixed with a binding agent.

18. (Previously presented) The vaccine according to Claim 17 wherein the binding agent comprises particulate feed.

19. (Previously presented) The vaccine according to Claim 17 wherein the binding agent comprises carboxymethylcellulose.

20. (Previously presented) The oral vaccine according to Claim 11 comprising immunologically effective dose of at least one of the proteins selected from

the group consisting of recombinant protein AHMA, and recombinant protein AHMA fragments.

21. (Previously presented) The oral vaccine according to Claim 1 further comprising an inactivated virus selected from a group consisting of guppy reovirus and guppy nervous necrosis virus.

22. (Previously presented) The oral vaccine according to Claim 1 further comprising bacterial antigens or killed bacteria selected from a group consisting of *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, and *Flexibacter columnaris*.

23. (Withdrawn) A method of making an oral vaccine comprising the steps of:

a) separately mixing a predetermined amount of at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the group consisting of isolated recombinant adhesin proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8, and immunogenic fragments thereof, either alone or in combination with at least one antigen selected from the group consisting of recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis*, guppy reovirus, guppy nervous necrosis virus, *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, and *Flexibacter columnaris* in a predetermined volume of at least one of water and saline;

b) vigorously mixing a pre-determined volume of organic oil with (a) to form an emulsion;

c) optionally, adding a binding agent to emulsion (b) with gentle stirring to obtain the consistency of a paste; and

d) optionally, adding particulate feed to (c) to obtain a particulate oral vaccine;

wherein said emulsion, paste, or particulate oral vaccine is orally suitable and said vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.

24. (Withdrawn) The method according to Claim 23 wherein the organic oil comprises palm oil.

25. (Withdrawn) The method according to Claim 23 wherein the binding agent comprises particulate feed.

26. (Withdrawn) The method according to Claim 23 wherein the binding agent comprises high viscosity carboxymethylcellulose.

27. (Previously presented) The vaccine prepared by the method according to Claim 48 wherein the computed dosage of recombinant AHMA in the vaccine ranges between 7  $\mu\text{g/g}$  and 150  $\mu\text{g/g}$  body weight of the recipient.

28. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of recombinant AHMA is between 15 $\mu$ g/g and 20  $\mu$ g/g body weight of the recipient.

29. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of recombinant AHMA is 17  $\mu$ g/g body weight of the recipient.

30. (Previously presented) The vaccine prepared by the method according to Claim 48 wherein the computed dosage of recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis* in the vaccine ranges between 7  $\mu$ g/g and 150 $\mu$ g/g body weight of the recipient.

31. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis* is between 15 $\mu$ g/g and 20  $\mu$ g/g body weight of the recipient.

32. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis* is 17  $\mu$ g/g body weight of the recipient.

33. (Previously presented) The vaccine prepared by the method according to Claim 48 wherein the computed dosage of at least one of viral proteins and inactivated virus in the vaccine ranges between  $10^3$  and  $10^6$  viral particles/g body weight of the recipient.

34. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of at least one of viral protein and inactivated virus is  $10^5$  viral particles/g body weight of the recipient.

35. (Previously presented) The vaccine prepared by the method according to Claim 48 wherein the computed dosage of at least one of inactivated bacterial and an equivalent amount of bacterial antigens in the vaccine ranges between  $10^5$  cfu/g and  $10^7$  cfu/g body weight of the recipient.

36. (Previously presented) The oral vaccine prepared by the method according to Claim 48 wherein the amount of at least one of inactivated bacteria and an equivalent amount of bacterial antigens in the vaccine is  $2.5 \times 10^6$  cfu/g body weight of the recipient.

37. (Withdrawn) A method of treating a species in need of such treatment against aquatic pathogens comprising administering an immunologically effective dose of the vaccine according to Claim 23.

38. (Withdrawn) A method according to Claim 37, wherein said animal is an aquatic species.

39. (Withdrawn) A method according to Claim 38, wherein the aquatic species is fish.

40. (Withdrawn) A method according to Claim 39, wherein the fish is a guppy.

41. (Withdrawn) A method according to Claim 39, wherein the fish is a blue gourami.

42. (Withdrawn) A method according to Claim 39, wherein the fish is a goldfish.

43. (Withdrawn) A fish immunized with the oral vaccine of Claim 23.

44. (Withdrawn) An edible product comprising fish immunized or treated with the vaccine according to Claim 25.

45. (Previously presented) The oral vaccine according to Claim 11 further comprising an inactivated virus selected from a group consisting of guppy reovirus and guppy nervous necrosis virus.



46. (Previously presented) The oral vaccine according to Claim 11 further comprising bacterial antigens or killed bacteria selected from a group consisting of *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, and *Flexibacter columnaris*.

47. (Previously presented) The oral vaccine according to Claim 21 further comprising bacterial antigens or killed bacteria selected from a group consisting of *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, and *Flexibacter columnaris*.

48. (Currently amended) An oral vaccine prepared by a method comprising the steps of:

a) separately mixing a ~~predetermined~~ an immunologically sufficient amount of at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the group consisting of isolated recombinant adhesin proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8, and ~~immunogenic fragments thereof~~, either alone or in combination with at least one antigen selected from the group consisting of recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis*, guppy reovirus, guppy nervous necrosis virus, *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Vibrio alginolyticus*, and *Flexibacter columnaris* in a predetermined volume of at least one of water and saline;

b) vigorously mixing a pre-determined volume of organic oil with (a) to form an emulsion;

c) optionally, adding a binding agent to emulsion (b) with gentle stirring to obtain the consistency of a paste; and

d) optionally, adding particulate feed to (c) to obtain a particulate oral vaccine;

wherein said emulsion, paste, or particulate oral vaccine is orally suitable and said vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.